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Nocturnal Visual Orientation in Flying Insects: A Benchmark for the Design of Vision-based Sensors in Micro-Aerial Vehicles

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14. ABSTRACT

In flying organisms such as insects, the sensory modalities that are available for flight control and navigation are more constrained than is the case in man-made aircraft. Insects do not carry radio communications equipment, radar, GPS, infrared sensors or large precision inertial systems, but rather get by with an assembly of conventional senses such as vision, mechanoreception, hearing and chemoreception. However, this sensor assembly, together with the information processing circuitry of the insect brain, is extremely miniaturized in comparison to any existing technical systems. Furthermore, each of these sensory systems has been under evolutionary selective pressure for the optimisation of its sensitivity and acuity. The visual sense, in particular, has often been adapted to the extreme limit of the physically possible.

Our research aimed to improve our knowledge in the general field of animal navigation and flight control, with a view towards applications in guided ammunition and Micro Aerial Vehicles (MAVs). Specifically, examined the physical limits of vision-based navigation and attitude control in insects. A first key topic addressed is the lower limit of light intensity at which insects are capable of using vision for obstacle detection and flight attitude control. A second topic is the elucidation of the neural principles that are responsible for this performance. A third topic is the lower limit to which polarised skylight is usable as a compass cue for navigation, both in terms of intensity and degree of polarisation. The identification of the critical parameters used by nocturnal insects for navigation and flight stabilisation in dim light will help us to identify the neural networks that are responsible. This in turn will allow the development of computational algorithms that analyse celestial cues for dim light navigation and flight stabilisation systems in machines.

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NOCTURNAL VISUAL ORIENTATION IN FLYING INSECTS: A BENCHMARK FOR THE DESIGN OF VISION-BASED SENSORS IN MICRO-AERIAL VEHICLES

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Principal Investigators: Drs. U. Homberg, G. Stange and E.J. Warrant

EXECUTIVE SUMMARY

In flying organisms such as insects, the sensory modalities that are available for flight control and navigation are more constrained than is the case in man-made aircraft. Insects do not carry radio communications equipment, radar, GPS, infrared sensors or large precision inertial systems, but rather get by with an assembly of conventional senses such as vision, mechanoreception, hearing and chemoreception. However, this sensor assembly, together with the information processing circuitry of the insect brain, is extremely miniaturized in comparison to any existing technical systems. Furthermore, each of these sensory systems has been under evolutionary selective pressure for the optimisation of its sensitivity and acuity. The visual sense, in particular, has often been adapted to the extreme limit of the physically possible.

One such adaptation is the ability to fly and navigate under a wide range of ambient light intensities, covering more that 8 orders of magnitude between full sunlight and the night sky. Some of our recent work is starting to show that low-light orientation occurs at intensities that are well below what was previously thought to be possible (Dacke et al., 2004, 2003; Greiner et al., 2005; Kelber et al., 2002; Warrant, 2004; Warrant et al., 2004). Nocturnal animals can see colour and negotiate dimly illuminated obstacles during flight. They can also navigate using learned terrestrial landmarks, the constellations of stars or the dim pattern of polarised light formed around the moon. The conclusion from these studies is clear: nocturnal habitats are just as rich in visual details as diurnal habitats are, and nocturnal animals have evolved visual systems capable of exploiting them. One of our model experimental animals in particular – the nocturnal tropical bee *Megalopta genalis* – has visual abilities in dim light that stagger the human observer. These bees forage in dense and extremely dark rainforests at night, and like their day-active relatives, are capable of learning visual landmarks and using them to find their way home after foraging trips. Home is a small hollowed-out stick camouflaged in the tangled rainforest undergrowth. This nest would be difficult enough to find during the day, but these bees find it at night when we ourselves see absolutely nothing at all apart from faint patches of sky visible through the canopy. We are only at the threshold of understanding how these bees achieve this.

Many insects detect the vector of polarisation of light from the sky and use this cue as a compass. When used together with one other parameter, such as a memory of the distance travelled (path integration), this compass is sufficient to enable return journeys over considerable distances. However, as the polarisation pattern originates from scattering of light in the atmosphere, it is subject to changes with solar and moon azimuth and to degradation by cloud cover. The work of one of the participating groups is starting to demonstrate that polarisation

patterns can be more degraded than previously thought, and, most importantly, that the dim polarisation patterns in the night sky are used for insect navigation.

Our research aimed to improve our knowledge in the general field of animal navigation and flight control, with a view towards applications in guided ammunition and Micro Aerial Vehicles (MAVs). Specifically, we proposed to research the physical limits of vision-based navigation and attitude control in insects. A first key topic addressed is the lower limit of light intensity at which insects are capable of using vision for obstacle detection and flight attitude control. A second topic is the elucidation of the neural principles that are responsible for this performance. A third topic is the lower limit to which polarised skylight is usable as a compass cue for navigation, both in terms of intensity and degree of polarisation. These three topics were explored as a collaborative effort by taking advantage of quite disparate and complementary animal models that have been developed in each of the participating labs.

Insect-inspired strategies for spatial orientation are likely to be considerably simpler than mechanisms found in vertebrates and humans. A combination of visual signals, including the distribution of light intensities and polarisation vector strengths sampled from large areas of the sky, ensure that the insect navigation system is robust over a wide range of sky conditions. The identification of the critical parameters used by nocturnal insects for navigation and flight stabilisation in dim light will help us to identify the neural networks that are responsible. This in turn will allow the development of computational algorithms that analyse celestial cues for dim light navigation and flight stabilisation systems in machines.

The outcome of this work will be of direct relevance for the design of MAVs: by identifying the limits to which biological systems are able to exploit dim light and noisy polarisation signals, and by determining the neural mechanisms that are used to maximise performance, we can provide designers of sensors and control systems with valuable information which could be implemented in artificial navigation and attitude control systems for use in very dim light.

TABLE OF CONTENTS

LIST OF FIGURES

I. GENERAL INTRODUCTION

Insects have the ability to fly and navigate under a wide range of ambient light intensities, covering more that 6 orders of magnitude between full sunlight and the night sky. Some of our recent work is starting to show that low-light orientation occurs at intensities that are well below what was previously thought to be possible (Dacke et al., 2003, 2004; Greiner et al., 2005; Kelber et al., 2002; Warrant, 2004; Warrant et al., 2004; Somanathan et al., 2008a, 2009; Warrant and Dacke, 2010a,b). Nocturnal animals can see colour and negotiate dimly illuminated obstacles during flight. They can also navigate using learned terrestrial landmarks, the constellations of stars or the dim pattern of polarised light formed around the moon. The conclusion from these studies is clear: nocturnal habitats are just as rich in visual details as diurnal habitats are, and nocturnal animals have evolved visual systems capable of exploiting them. One of our model experimental animals in particular – the nocturnal tropical bee *Megalopta genalis* – has visual abilities in dim light that stagger the human observer. These bees forage in dense and extremely dark rainforests at night, and like their day-active relatives, are capable of learning visual landmarks and use them to find their way home after foraging trips. Home is a small hollowed-out stick camouflaged in the tangled rainforest undergrowth. This nest would be difficult enough to find during the day, but these bees find it at night when we ourselves see absolutely nothing at all apart from faint patches of sky visible through the canopy. We are only at the threshold of understanding how these bees achieve this, and two of the participating groups (Warrant's and Stange's) have been performing behavioural studies on freely flying bees in a Panamanian rainforest, and electrophysiological studies in their compound eyes and ocelli to try and elucidate the neural mechanisms responsible. For the electrophysiological studies, a new two-dimensional spatiotemporal white-noise stimulation system has been developed.

Many insects detect the vector of polarization of light from the sky and use this cue as a compass. When used together with one other parameter, such as a memory of the distance travelled (path integration), this compass is sufficient to enable return journeys over considerable distances. However, as the polarization pattern originates from scattering of light in the atmosphere, it is subject to changes with solar and moon azimuth and to degradation by cloud cover. Warrant's group has been demonstrating that dung beetles can accurately navigate using the dim polarization pattern produced around the moon. We have previously found that diurnal species can navigate using the bright polarization pattern produced around the sun, and here we report that if forced these species are even capable of navigating at night using the polarization pattern produced around the moon, showing that despite being diurnal their superposition eyes are sensitive enough to analyze polarized light at night. The Homberg group is continuing their investigations of the responses of polarization-sensitive neurons in the central complex of solitary and gregarious locusts at different light levels, and has in addition begun studies of the properties of the photoreceptors of the dorsal rim area. They have discovered that the photoreceptors and interneurons of solitary locusts are considerably more sensitive at night than those of gregarious forms and that the interneurons are considerably more sensitive than the photoreceptors that feed them, suggesting the presence of summation mechanisms. Within the Stange group, Josh van Kleef has now fully tested a new spatiotemporal white noise stimulus that promised to open up many new avenues of research, particularly regarding the measurement of the spatial and temporal receptive fields of visual cells and the calculation of their information rates.

This report will highlight the accomplishments of the three groups in their investigations of nocturnal and dim light vision

II. PROGRESS REPORT WARRANT

II.1 INTRODUCTION

In our part of the night vision project, we investigated visual performance in nocturnal bees and dung beetles, as well as in closely related diurnal species for comparison. We studied the behaviour of insects when they orient in dim light, and are using electrophysiology, histology and modelling to study the optics and physiology of the compound eyes and ocelli. The aim of these studies were to understand how nocturnal insects are able to navigate in very dim light, and which neural mechanisms are responsible.

This work is led by Eric Warrant and Marie Dacke, and is performed together with two postdoctoral fellows – Jochen Smolka (who began in January 2010) and Emily Baird (who started in February 2008). Josh van Kleef (from the Stange group) is also involved via his development of a new two-dimensional spatiotemporal white-noise stimulation system. This stimulus system will be used to study the information capacities and visual field properties of photoreceptors and second order cells in the ocelli and compound eyes of nocturnal bees. This work will be done in a new purpose-built electrophysiology lab at the Smithsonian Tropical Research Institute in Panama City. Due to the difficulty in exporting/importing bees and then keeping them alive in Lund for more than a couple of weeks, these demanding experiments will be performed in Panama where we have access to a virtually endless supply of bees.

One of our chief model animals – the nocturnal halictid bee *Megalopta genalis* – has yielded a large number of important results that have helped to shed light on the how the optics and physiology of the compound eyes have been adapted for night vision (Warrant et al., 2004; Greiner et al., 2004a; Frederiksen et al., 2008). These studies all point to the necessity of a neural summation mechanism at an early stage of visual processing, and histological (Greiner et al., 2004b; Greiner et al., 2005), electrophysiological (Frederiksen et al., 2008) and theoretical (Theobald et al., 2006) studies all point to the lamina as the likely location for this summation. During the fourth 6 months of this project period we continued our electrophysiological studies of the large monopolar cells (LMCs) of the lamina that we believe are responsible for this summation. However, the project has continued to prove to be extremely difficult, and we have had limited success (partly also due to the difficulty of getting bees to Lund). These experiments will thus be moved to Panama for the reasons outlined above. As mentioned in the previous report, in Lund we are now about to attempt the same experiments in other species of nocturnal insects, including nocturnal hawkmoths and nocturnal crane flies. To this end, we have built a brand new lab designed for stimulation of higher visual centres in insects using patterns generated on a very fast monitor. This new lab was built in collaboration with Prof. David O'Carroll (University of Adelaide) who has been in Lund on sabbatical since July.

We again made our annual three-week field trip to Panama during March where we continued our behavioural experiments to explore the visual abilities of freely-flying *Megalopta* in extremely dim light. In these experiments we sought to determine (1) the ability of *Megalopta* to approach and land on its nest entrance at night (using high-speed filming in bright infrared light), (2) whether *Megalopta* uses optic flow cues to stabilize its flight trajectory at night (done by forcing them to fly through square-sectioned Perspex tunnels lined with black-and-white patterns and filming their flight trajectories from below in infrared light), and (3) whether *Megalopta* uses mechanosensory cues (in addition to vision) to localize and land upon its nest (done by placing an invisible piece of sapphire glass a few centimeters in front of the nest). Together these experiments all indicate that *Megalopta* uses vision and optic flow cues to control flight and to land in dim light. The fact that we can now say unequivocally that *Megalopta* uses optic flow to

stabilize its flight trajectory is a major advance in our understanding of nocturnal visual performance in insects and opens a number of new lines of research. Some of the results of the first experiments on landing precision and the use of optic flow in darkness were detailed in the last report. Since that report, more data has been analyzed and new experiments on diurnal bumblebees have been performed as a comparison.

Another one of our model animals – nocturnal dung beetles – has also yielded many further results since the last report. As a result of field-trips to South Africa in 2009 and 2010, we have now completed our studies to determine the ability of dung beetles to orient to stars. As we mentioned in the last report, we made the surprising discovery that one species of nocturnal dung beetle – *Scarabaeus satyris* – orients using the Milky Way. In contrast, we have previously found that diurnal species can navigate using the bright polarization pattern produced around the sun, Here we report that if forced these species are even capable of navigating at night using the polarization pattern produced around the moon, showing that despite being diurnal their superposition eyes are sensitive enough to analyze polarized light at night. This remarkable result, and the methodology used, is detailed below.

II.2 METHODS, ASSUMPTIONS, AND PROCEDURES

II.2.1 Topic 1: Low-light vision in the halictid bee compound eye

Behaviour

The site for all behavioural experiments was in the rainforests of Barro Colorado Island, a tropical research station in the Panama Canal administered by the Smithsonian Tropical Research Institute.

Dim light landing precision in Megalopta. Executing an accurate landing is one of the most demanding behavioural tasks a flying insect is required to perform. The difficulty of this task is further increased for the nocturnal sweat bee, *Megalopta genalis*, who performs this behaviour under extremely dim light conditions using apposition eyes – normally only found in diurnal insects.

To investigate the accuracy with which *Megalopta* is able to land under dim light conditions, we recorded the flights of bees in the final stage of approach and landing under light intensities that varied over 6 orders of magnitude. On our previous field trip to Panama in 2009, we performed a pilot study where we recorded the flights of *Megalopta* approaching natural nest sticks. One problem with using natural nest sticks for this type of investigation is the variance that occurs between nests, especially in terms of wood colour, stick size, hole size and hole position. These sources of variation would provide approaching bees with different visual cues, which would affect the interpretation of our results. To minimise the variation between different nests, we covered the end of the nest stick with a 5.5 cm diameter Perspex disk with a 5 mm diameter hole drilled in the centre. By covering the nest with this disk, we could create a 'standardised' nest entrance that would allow us to more accurately compare landings between different individuals from different nests. In addition, using the Perspex nest entrances allowed us to test the effect of differences in contrast on landing accuracy by covering the disk with either black or white paper.

The nest sticks were placed at the end of a flight tunnel so that we could record both the approach and the final landing phase (Figure 1). The approach was filmed over a distance of 50 cm and was filmed using a handycam recording at 25 Hz, the final landing phase was filmed using a high speed camera recording at 300 Hz over a distance of 10 cm.

Role of the visual detection of optic flow in flight control in Megalopta. To investigate the mechanisms of flight control in *Megalopta*, we trained bees to fly through specially designed experimental tunnels and recorded their flight trajectories. The experimental tunnel consisted of a Perspex rectangular tube 15 cm wide x 15 cm high x 50 cm long. The end of the nest stick protruded 5 cm into the end of the tunnel so that to exit, or enter the nest, a bee was required to fly through the tunnel. Visual textures were affixed to the inner walls of the tunnel such that the pattern was visible to the bee flying in the tunnel; the flights of bees flying to and from the nest were recorded using a video camera placed underneath the tunnel. Flights to the nest stick were recorded when the pattern on the walls displayed either a randomised chequerboard pattern, or a horizontal stripe pattern.

Figure 1: The experimental setup used to investigate dim light landing precision in Megalopta. The nest stick was covered with a Perspex disk that was either black or white. The end of the nest stick was placed inside a flight tunnel. Approaches in the flight tunnel were filmed over 50 cm using a handycam placed under the flight tunnel. The final landing phase was recorded from the side over a distance of 10 cm using a

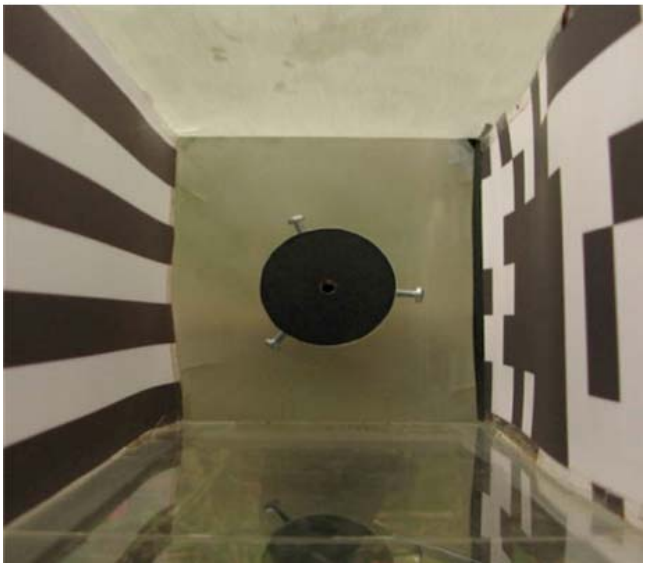

Figure 2. The flight tunnel used to investigate how *Megalopta* uses visual information to fly in the centre of the tunnel at different light intensities. One wall of the tunnel displays a chequerboard pattern, which provides strong optic flow cues, whilst the other displays a horizontal stripe pattern, which provides only weak optic flow cues. If the bees are using lateral optic flow cues to centre in the tunnel, they should fly closer to the wall with the horizontal stripes.

These experiments were started on our field trip to Panama in 2009. We decided to repeat them again this year to increase the data set and the number of individuals that we test. This will

provide us with more accurate data on how visual information is being used to control flight in dim light.

In addition to repeating the experiments that we performed last year, we also investigated how *Megalopta* use optic flow to fly in the centre of the tunnel by placing a checkerboard pattern on one wall and a horizontal stripe pattern on the other (Figure 2). If *Megalopta* use optic flow to centre in the tunnel, they will try to balance the rate of optic flow perceived in each eye. When one of the walls provides very little optic flow information, which is the case for the horizontal stripe pattern, we expect that the bees will fly closer to that wall in an attempt to balance the different rates of optic flow perceived in each eye.

III.3.2 Topic 2: Low-light polarization vision in the dung beetle compound eye

Nocturnal dung beetles remain the only insects that have been demonstrated to use the polarisation pattern around the moon and even the stars as directional cues for orientation. Supposedly, their highly adapted visual systems – with larger lenses, wider and longer rhabdoms than their diurnal relatives and a tracheal tapetum (Figure 3) – enable them to perform this difficult task.

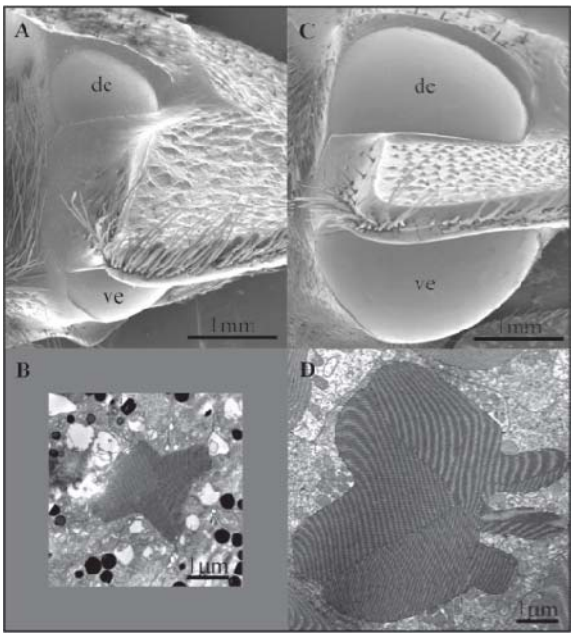

Figure 3: Comparison of the compound eyes (A,C) and light-absorbing rhabdoms (B,D) of the day-active *S. nigroaeneus* (A,B) and the night-active *S. satyrus*. Larger eyes and rhabdoms should give *S. satyrus* higher light-sensitivity at night.

To investigate how important these specialisations are for nocturnal orientation and navigation, we compared the orientation performance of the nocturnal *Scarabaeus satyrus* and the exclusively diurnal *S. nigroaeneus* in their natural habitat in South Africa under four different light conditions at night. The diurnal *S. nigroaeneus* can be enticed to roll at night, despite this being highly unusual behaviour for them. For each condition, we placed 10-20 beetles of each species with their ball in a circular arena (diameter 3 m) and filmed them from above under infrared illumination. The tracks were reconstructed from the videos and their straightness evaluated from the path length. We ignored the initial activity (less than 10 cm from the centre), so a perfectly straight path would be 140 cm long.

II.3 RESULTS AND DISCUSSION

Two important field trips were conducted earlier this year to collect animals and to perform experiments: (1) to study dung beetle behaviour in South Africa (February 2010), and (2) to collect and study nocturnal sweat bees in Panama (March 2010). Our two postdoctoral fellows (Eva Kreiss (who finished in May 2010) and Emily Baird) conducted experiments in various parts of both projects. One paper has now been published (Baird et al. 2010), one paper is in press in the *Proceedings of the Royal Society* from our work in South Africa (Dacke et al. 2010) and one has been submitted to *Biology Letters* (Baird et al. 2011). Further preliminary results from some of the behavioural studies in Panama are presented below. As mentioned in the last report, Richard Berry, from the Stange group, finished a major study on the physiology and optics of nocturnal bee ocelli. This work is about to be re-submitted to the *Journal of Experimental Biology* after being re-reviewed. Unfortunately, our continued attempts to record from the lamina monopolar cells of nocturnal bees remains problematic. To solve this, we are moving these experiments to a new electrophysiology lab at the Smithsonian Tropical Research Institute in Panama City where we have unlimited access to bees (which has been one of the major problems with this difficult project).

II.3.1 Topic 1: Low-light vision in the halictid bee compound eye

Dim light landing precision in Megalopta

Figure 4: *Megalopta* extending her legs in preparation for landing on her nest. The leg extension is a stereotyped behaviour that can be used to assess landing accuracy under different light intensities.

As a bee approaches her nest stick, she extends her legs in preparation for landing in a stereotypical manner, making it a useful parameter to compare across different landings (Figure 4). In our fourth report, we described the results of our experiments that were conducted in Panama in 2009. These results showed that leg extension occurs at a constant 'time-to-contact' – i.e. the time between leg extension and contact with the nest stick is the same. This result provides a strong indication that *Megalopta* rely on optic flow cues to initiate leg extension. This is because leg extension at a constant time-to-contact would be achieved by monitoring the rate of expanding optic flow generated by the nest stick as it is approached and initiating leg extension when this rate of optic flow exceeds a certain threshold level. We have now fully analysed the

results of our most recent experiments (conducted in March 2010). In accordance with the findings of our previous study (conducted in 2009), these results show that the time between leg extension and contact with the surface during landing is not dependent upon light intensity (Figure 5a). We also find that the distance from the surface at which leg extension is initiated varies very little and is not affected by light intensity (Figure 5b), indicating that the speed at which *Megalopta* approaches the nest stick during landing is relatively constant. Our findings indicate that light intensity does not compromise the accuracy with which *Megalopta* approach and land on their nest stick.

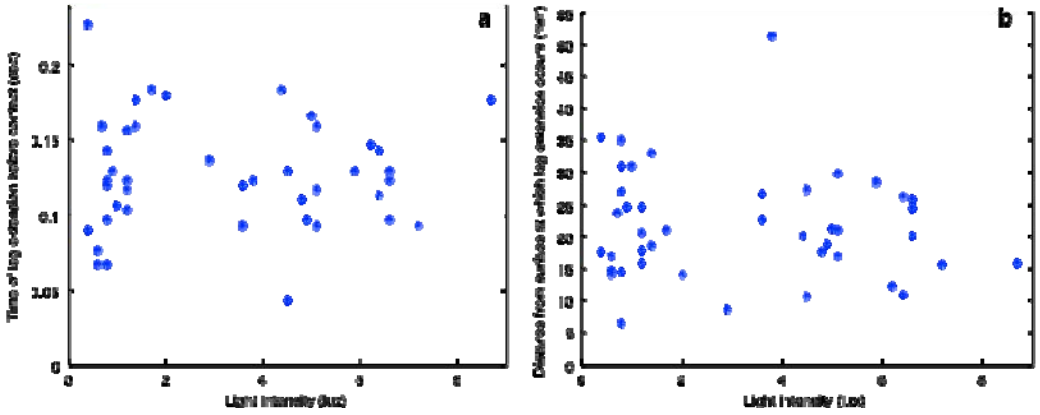

Figure 5: The effect of light intensity on the initiation of leg extension in *Megalopta*. The effect of light intensity on landing accuracy as measured by either the time (a) or the distance (b) between leg extension and contact with the landing surface. There does not appear to be a correlation in either case.

Role of the visual detection of optic flow in flight control in Megalopta

Our analysis of the data reveals some intriguing results. We find that flight speed increases as the amount of horizontal (front-to-back) optic flow cues in the tunnel decrease (Figure 6a). Although earlier studies on honeybees have used similar pattern configurations to investigate the role of horizontal optic flow on centring, the effect of an asymmetric pattern scheme (i.e. a chequerboard pattern on one tunnel wall and horizontal stripes on the other) on flight speed is not known.

In our analysis of the centring response in *Megalopta*, we found that asymmetries in the amount of horizontal optic flow available in the tunnel did not affect the ability of the bees to fly in the centre of the tunnel (Check/Stripe condition, Figure 6b), contrary to the results observed in honeybees. Combined with the flight speed data analysis, these results suggest that *Megalopta* are using optic flow for flight control in a different way to day-active insects such as the honeybee. In the absence of flight speed data from a day active insect under the pattern configurations used in our experiments, however, the extent to which the behaviours observed in these two species diverge is not clear.

To gain a better understanding of the differences between the visually guided flight control behaviours observed in the nocturnal *Megalopta* and those observed in day-active hymenopterans,

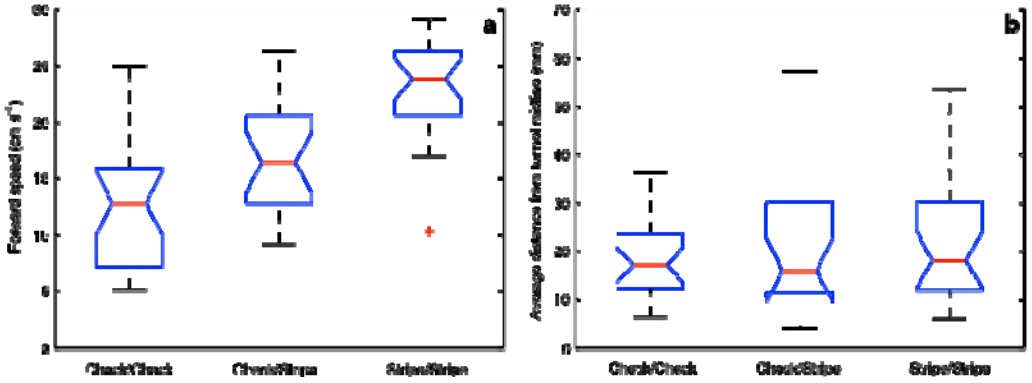

Figure 6: The effect of horizontal optic flow on flight speed (a) and centring (b) in *Megalopta*.

we repeated the experiments described above on bumblebees. In a recent publication (based on work supported by the EOARD) we showed that, like honeybees, bumblebees use optic flow to control flight speed. Moreover, the visual systems of the large bumblebee foragers used in our experiments are of a similar size to those of *Megalopta*. Bumblebees therefore provide a good diurnal comparison for optic flow flight control behaviour in *Megalopta*. Repeating this experiment using the exact same experimental paradigm and set-up that was used for *Megalopta*, we are able to directly compare the visually guided flight control behaviours observed in both species and to identify any differences that may exist.

The results of this experiment reveal that, when the horizontal optic flow cues on one wall of the tunnel are removed (Check/Stripe condition in Figure 7), flight speed in bumblebees remains the same as when both walls display strong horizontal visual cues (Figure 7a). Moreover, bumblebees no longer fly in the centre of the tunnel when horizontal visual cues are removed from one wall (Figure 7b). These results are unlike those observed in *Megalopta* and provide strong support for our hypothesis that *Megalopta* have developed different strategies for using optic flow for flight speed control, which may be more effective for navigating under dim light conditions.

One question that arises from our investigations is whether the strategy adopted by *Megalopta* for using optic flow is conserved in insects that fly in dim light, or whether it is a strategy unique to *Megalopta*. To help us answer this question, we will investigate the effect of horizontal optic flow on flight control in an insect that flies under both bright, daylight conditions, and under very dim light conditions. The common wasp, *Vespula vulgaris*, is a suitable species for answering this question as they forage under both daylight and dim light conditions. Our future investigations will focus on whether wasps are affected by changes in the amount of horizontal motion cues available in the experimental tunnel under different light intensities and whether the strategy changes as light intensity decreases.

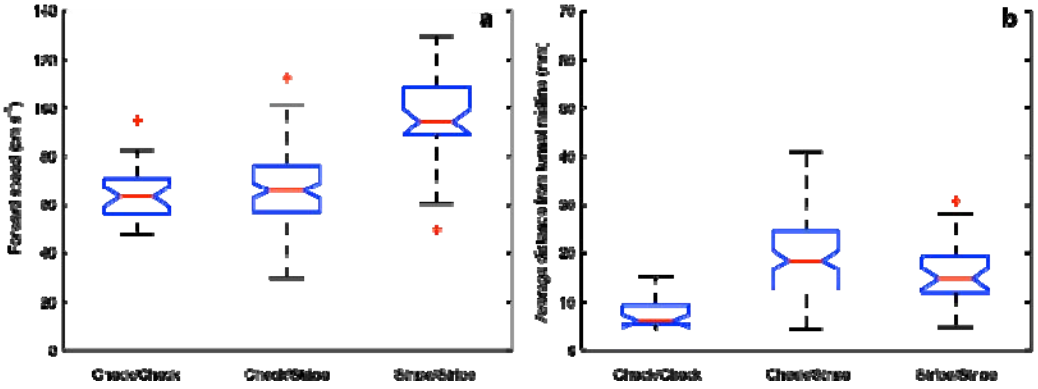

Figure 7: The effect of horizontal optic flow on flight speed (a) and centring (b) in bumblebees.

III.3.2 Topic 2: Low-light polarization vision in the dung beetle compound eye

With the aid of an artificial light or with clear view of a quarter moon, neither species has problems keeping a straight course (Figure 8). Interestingly, both species preferentially roll towards the bright artificial light. As the beetles roll backwards, this puts them into the shadow of their own ball with their back towards the light. Under the quarter moon, on the other hand, *S. nigroaeneus* prefer to roll away from the light, thus keeping the much dimmer light source in the centre of their visual field.

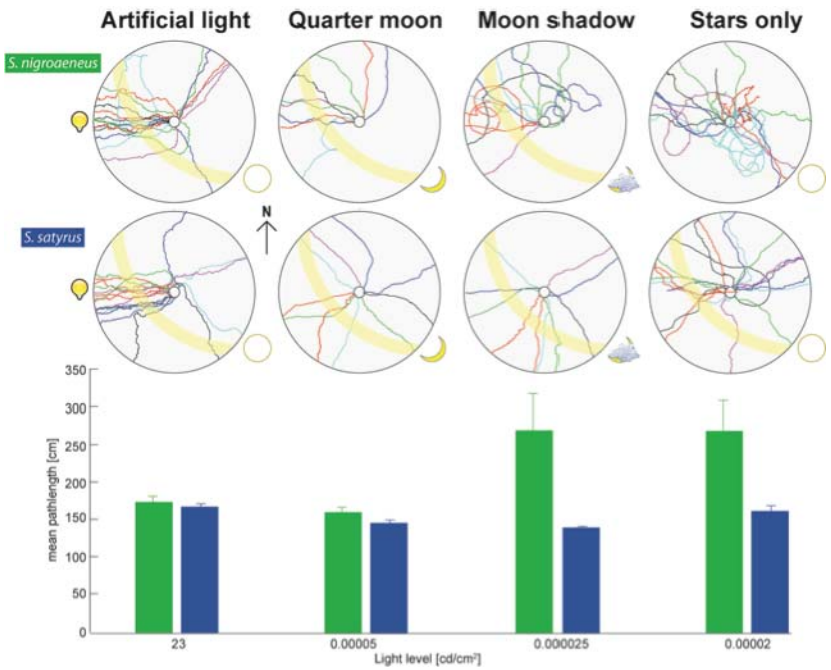

Figure 8: Rolling paths of *S. nigroaeneus* and *S. satyrus* under different light conditions in their natural habitat. While the moon or an artificial light source is present there is no difference in performance between the two species. Even under the light of the Milky Way (position indicated by *yellow bar*), a large number of day-active beetles orient successfully.

Under a quarter moon shaded by a board and on a moonless night with only the stars to guide them, some of the diurnal beetles could not keep their direction anymore and started rolling in circles. Surprisingly, however, the majority of both nocturnal and diurnal beetles can still use the dim light of the Milky Way as an orientation cue (see previous report).

We confirmed the ability of *S. nigroaeneus* to orient under low light levels by presenting both species with an adjustable point light source in the laboratory (Figure 9). When the intensity was reduced, tracks became longer, but we found no significant difference between the performances of the two species. These results indicate that the advantage of the nocturnal eye design lies in its ability to perform well under diffuse illumination, e.g. on cloudy or moonless nights.

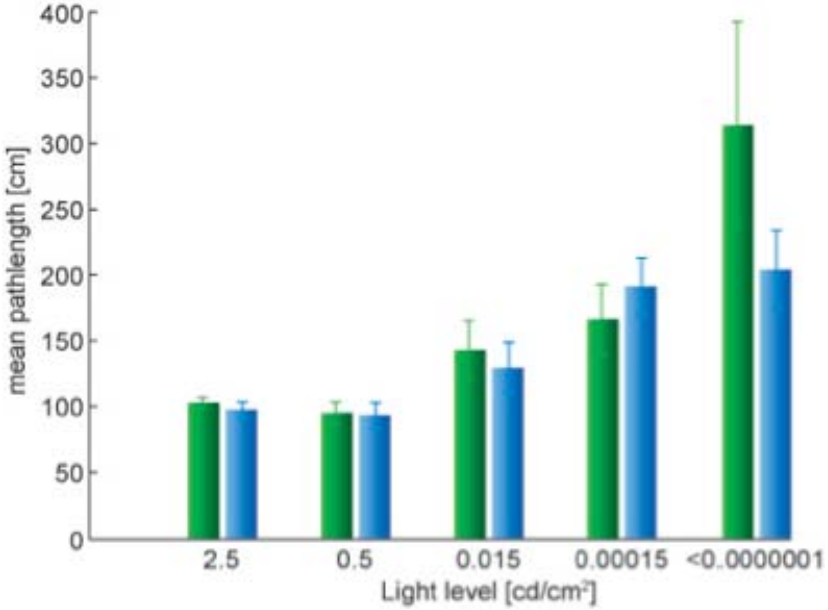

Figure 9: Orientation performance of *S. nigroaeneus* (green) and *S. satyrus* (blue) under a point light source of adjustable intensity in the laboratory. Under almost all conditions there is no difference between species. Only in the dark, *S. satyrus*' paths are shorter, likely due to their larger size and higher rolling speed.

III. PROGRESS REPORT STANGE

III.1 INTRODUCTION

Within the overall framework of the night vision project, our objective has been to provide information on the nocturnal habits and visual processing of the dragonfly, an animal model that has so far been considered an extreme example of adaptation to a diurnal lifestyle. With the recent retirement of Gert Stange (at the end of 2009), this part of the research program has now closed. A second part of our work has also been to elucidate the visual performance of ocelli in the nocturnal bee *Megalopta* – this work will soon be published in the *Journal of Experimental Biology* (Berry et al. 2010). A third part of our work has been to develop a new two-dimensional spatiotemporal white noise display to measure the spatial and temporal receptive fields of visual cells in nocturnal insects and to calculate their information rates (performed by Josh van Kleef). This third part of the project is reported here.

The nocturnal bee *Megalopta genalis* is able to navigate through the dark rainforest canopy despite having an eye design typical of a day-active insect (Warrant et al., 2004). It is thought that they achieve this remarkable feat by spatially integrating light information via neural mechanisms (Warrant et al., 1996). Spatial integration increases the reliability with which a neuron can signal visual information while sacrificing the spatial resolution of the neuron.

Supporting this hypothesis, anatomical work has shown that greater spatial summation occurs in laminar neurons of *Megalopta* (Greiner et al., 2004). This part of the project is aimed at directly measuring the spatial receptive fields (RFs) of *Megalopta* lamina neurons at different times after stimulation. These spatiotemporal RFs provide a map of how a neuron integrates light and can be used to probe changes in the spatial and temporal properties of cells as light levels fall.

III.2 METHODS, ASSUMPTIONS, AND PROCEDURES

III.2.1 Topic 1: Low-light vision in the halictid bee compound eye.

We preivously developed an electrophysiological data collection system which is synchronized with a 120 Hz CRT. The CRT visual stimulus, when combined with a cover and neutral density filters, is capable of displaying very low intensities (Fig. 10A). Experiments consist of recording the electrical response of a neuron with an intracellular glass electrode while a random stimulus is presented on the CRT in a dark room. By combining the stimulus and response we can estimate the spatial receptive fields.

RF estimation is a two-stage process (see Fig. 10B). Initially, the entire screen will be modulated with a random stimulus at a fairly coarse resolution (Step 1). Using a fast online analysis the data recorded in step 1 will be used to locate the approximate position and size of the RF of the neuron from which we are recording. Using the information obtained from the first step a smaller rectangle of white noise that covers the receptive field at much higher resolution will be used to map the RF in more detail.

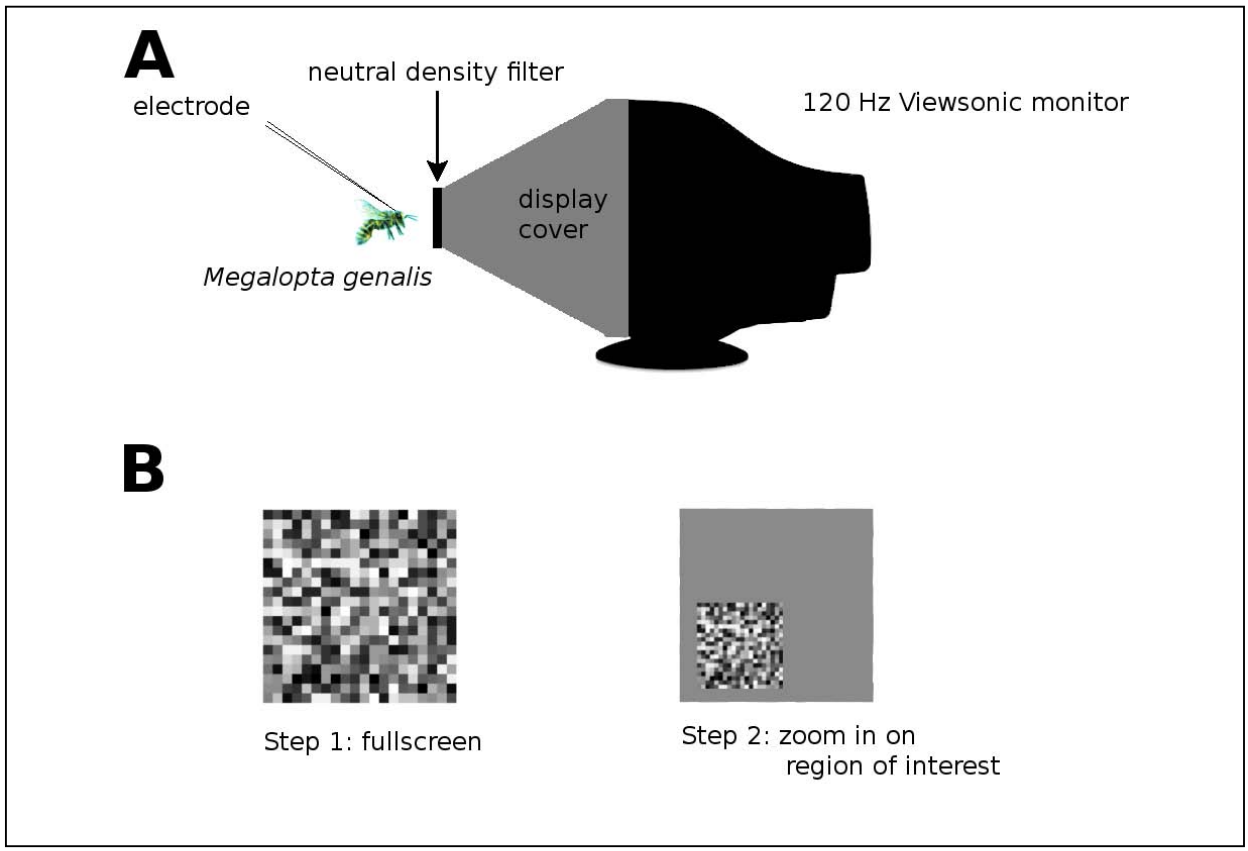

Figure 10: A new two-dimensional spatiotemporal white noise stimulus for recording the spatial and temporal receptive fields of visual neurons. A. The experimental arrangement showing the position of a nocturnal bee relative to the monitor. B. The stimulus paradigm.

III.3 RESULTS AND DISCUSSION

III.3.1 Topic 1: Low-light vision in the halictid bee compound eye.

Although *Megalopta genalis* have been difficult to obtain, some thirty *Megalopta ecquadoria* bees were obtained for recording. These bees are closely related to *Megalopta genalis* and share the same nocturnal lifestyle and habitat. Unfortunately, no laminar cells were obtained (these have proven very difficult to record from, as mentioned above). However, 10 photoreceptors were recorded and these were used to test the system. An example is shown in Fig. 11. The top panel (Fig. 11A) shows the photoreceptor responses to a 'rough' stimulus (coloured traces) and the spatial RF (red shading) obtained from these responses. The bottom panel (Fig. 11B) shows the photoreceptor responses to a 'finer' stimulus (coloured traces) and the spatial RF (red shading) obtained from these responses.

One can see that the $7 \times 40 = 280$ seconds of white noise is not quite sufficient to produce smooth RFs when the 'finer' resolution is used. Nonetheless, in both cases our technique produces acceptable RFs; which is remarkable given the small amount of correlation seen in the raw data between trials (see coloured traces).

IV. PROGRESS REPORT HOMBERG

IV.1 INTRODUCTION

Research in our group is aimed at understanding the neural mechanisms underlying sky compass navigation in insects. Our main experimental model system is the desert locust *Schistocerca gregaria*. *Schistocerca* occurs in a gregarious and a solitarious form that differ substantially in coloration, behaviour and endocrinology (Uvarov, 1966; Pener, 1991; Simpson et al., 1999). At high population densities, animals crowd together and perform long-range migrations as larval hopper bands or flying swarms of adult animals, presumably driven by search for food and new breeding grounds (Kennedy, 1951; Baker, 1978). At low population densities, desert locusts occur in the solitarious form and animals actively avoid each other (Simpson et al., 1999). While gregarious locusts migrate exclusively during the day, solitarious locusts preferentially migrate during the night (Waloff, 1963; Roffey, 1963; Riley and Reynolds, 1986). This phenotypic plasticity offers the unique opportunity to study adaptive changes in the orientation mechanisms between diurnal and nocturnal migrants of the same species.

Locusts have a well-developed polarization vision system (Homberg et al., 2008). They perceive the sky polarization pattern through photoreceptors in a highly specialized dorsal rim area of their compound eyes (Homberg and Paech, 2002; Mappes and Homberg, 2004). Tracing studies and intracellular recordings combined with single-cell dye injections revealed the central processing stages for polarized light in the locust brain (Homberg et al., 2003). They include the anterior lobe of the lobula in the optic lobe and the anterior optic tubercle and the central complex in the central brain (Heinze and Homberg, 2007, 2009). In the anterior optic tubercle, we studied especially three types of interneuron. The lobula-tubercle neuron 1 (LoTu1, a single neuron per hemisphere), and the tubercle-tubercle neurons (TuTu1, a pair of neurons in each hemisphere) connect the anterior optic tubercles of both brain hemispheres. A third type, termed tubercle-lateral accessory lobe neurons (TuLAL1a, about 20-30 per hemisphere) connect the tubercle to neurons of the central complex (Pfeiffer et al., 2005; Kinoshita et al., 2007; Pfeiffer and Homberg, 2007). The anterior optic tubercle neurons respond to (i) polarized-light stimuli perceived by the polarization-sensitive dorsal rim area of the eye and (ii) to colour stimuli perceived by dorso-lateral parts of the eye in a way suggesting that they combine inputs from the polarization pattern of the sky and the chromatic contrast of the sky for increased robustness in signalling of azimuthal directions (Pfeiffer and Homberg, 2007).

A major goal within our AFOSR grant is to compare the physiological properties of neurons of the anterior optic tubercle in gregarious and solitarious locusts, with particular focus on the absolute sensitivity of these cell types. During the last six months, we added substantial data on the physiology of TuTu1- and LoTu1 neurons and compared the absolute sensitivity of both cell types in gregarious and solitarious animals recorded during the day and at night (topic 8). These experiments were performed by Basil el Jundi, a PhD student in the laboratory. In addition, a second graduate student, Fabian Schmeling, has established a new experimental station for intracellular recording from photoreceptors of gregarious and solitarious locusts and has provided first data on the spectral sensitivity and intensity-response relationship of photoreceptors of the dorsal rim area of the eye (topic 8).

IV.2 METHODS, ASSUMPTIONS, AND PROCEDURES

IV.2.1 Topic 8: Performance of interneurons of the locust sky navigation system at low light levels

Recordings from dorsal rim area photoreceptors

A new experimental stand was established to perform intracellular recordings from dorsal rim photoreceptors of the locust eye. A graduate student, Fabian Schmeling, spent three months in the laboratory of Dr. Kentaro Arikawa and Dr. Michiyo Kinoshita to become familiar with insect photoreceptor recordings and has now started to do experiments in locusts. Recordings were obtained with glass micropipettes filled with 3 M KCl. Visual stimuli were provided with a xenon arc lamp, a monochromator, a set of neutral density filters, and a neutral density wedge. Light was delivered to the locust through a quartz light guide (visual angle 2.6°). To test for spectral sensitivity, monochromatic light flashes (duration 500 ms) of equal photon flux (3×10^{12} photons s^{-1} cm^{-2}; bandwidth 15 nm) were given in 20 nm steps between 310 and 630 nm. Subsequently, response intensity (R/log I) curves were determined at the peak wavelength with light flashes of increasing light intensity (Log I = 0: 2.2×10^{13} photons s^{-1} cm^{-2}). Only recordings with peak depolarizations of at least 30 mV were used for further evaluation. Spectral sensitivity curves were calculated by taking into account the Naka-Rushton equation of intensity-response curves.

Recordings from interneurons of the anterior optic tubercle

Polarization-sensitive neurons of the anterior optic tubercle were analyzed through intracellular recordings, using glass micropipettes filled with Neurobiotin. For anatomical identification of the recorded neuron type, Neurobiotin was injected into the cell after recording. To reveal possible differences in the sensitivity of these neurons in gregarious and solitarious locusts we analyzed the bilateral extension of the receptive fields and absolute sensitivities in animals from both morphs. The center and bilateral size of the receptive fields were determined by stimulating animals during the recording from different elevations along the right-left meridian with linearly polarized monochromatic blue light (450 nm; max. photon flux 6.98×10^{13} photons cm^{-2} s^{-1}; angular extent at locust eye ~4.7°). Polarized light was produced by passing light of a xenon lamp (XBO 150W) through a polarizer (Polaroid, HNP'B). During stimulation, the polarizer was rotated in clockwise (0°-360°) and counterclockwise (360°-0°) direction with a rotating speed of 30 °/s.

To quantify the response strength of a neuron to polarized light given at different positions of the visual field, the strength of frequency modulation during rotation of the polarizer, termed response amplitude *R*, was calculated as described by Labhart (1996). Briefly, each filter rotation was divided into 20° bins. The mean spiking activity over all bins was calculated as well as the frequency within each bin. The summed absolute difference between the mean frequency and the individual frequencies was defined as the *R*-value of that polarized light response and reflected the magnitude of frequency modulation during rotation of the polarizer. The elevation along the right-left meridian, at which the neuron showed the highest *R* value, was taken as the center of the receptive field. Response-intensity functions of the neurons were determined by reducing the light intensity in the center of the receptive field in logarithmic steps through a set of neutral density filters. The response strength of the neurons during stimulation with different intensities of polarized light was determined again by calculating the response value *R*. Likewise, variability in background spiking was calculated as the *R* value in parts of the spike train without stimulation.

IV.3 Results and Discussion

IV.3.1. Topic 8: Performance of interneurons of the locust sky navigation system at low light levels

Recordings from dorsal rim area photoreceptors

Recordings from photoreceptors of the dorsal rim area of the locust eye are now routinely performed by Fabian Schmeling. In accordance with the spectral sensitivity of polarization-sensitive interneurons (Kinoshita et al. 2007) photoreceptors recorded in the dorsal rim area are blue sensitive with maximum sensitivity at 450 nm (Fig. 12A). Response-intensity functions show the typical sigmoid shape (Fig. 12B). All data were obtained from gregarious animals and fit well to a previous account by Eggers and Gewecke (1993) on dorsal rim photoreceptors in desert locusts. In future experiments, the data will be complimented with recordings from all photoreceptor types in gregarious animals, studies on the acceptance angle of dorsal rim photoreceptors and their polarization-sensitivity. Parallel experiments will provide corresponding data for solitarious locusts.

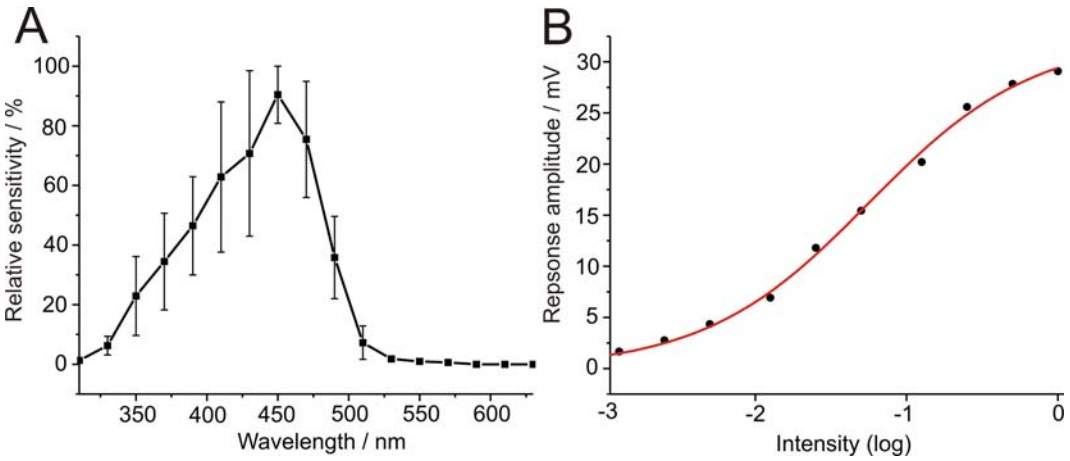

Figure 12: (A) Spectral sensitivity curve of blue receptors in the dorsal rim area of gregarious locusts. Data are based on 10 spectral tests from 2 photoreceptor cells. **(B)** Intensity-response curve at 450 nm wavelength. Log I = 0: 2.2×10^{13} photons s^{-1} cm^{-2}. The Naka-Rushton fit shows a sigmoid shape of the curve.

Recordings from interneurons of the anterior optic tubercle

Neurons of the anterior optic tubercle were analyzed in 75 intracellular recordings. Neurons that were sensitive to polarized light typically responded with a sinusoidal modulation of firing rate during stimulation with a rotating polarizer (Figs. 13A; 14A; 15A). The *E*-vector orientation, at which maximum spiking activity occurred, was defined as the preferred *E*-vector orientation of the neuron and was termed Φ_{max} (Figs. 13B; 14B; 15B). The intertubercle neuron TuTu1 was analyzed in 29 experiments (Fig. 13). Sixteen of these experiments were performed in gregarious animals. TuTu1 neurons of gregarious locusts had a background activity of 26.6 ± 2.7 (mean ± SE) impulses per second and a background variability of 39.9 ± 3.9 (mean ± SE). The distribution of Φ_{max} orientations, determined in 13 recordings, is shown in Fig. 2D (left circular plot). Φ_{max} values of the analyzed gregarious TuTu1 neurons are clustered around three *E*-vector orientations, at 40°, 145°, and 175°. The receptive field of the TuTu1 neurons of gregarious locusts is about 110° wide at half maximum response amplitude and is centered at an elevation of 60° contralaterally.

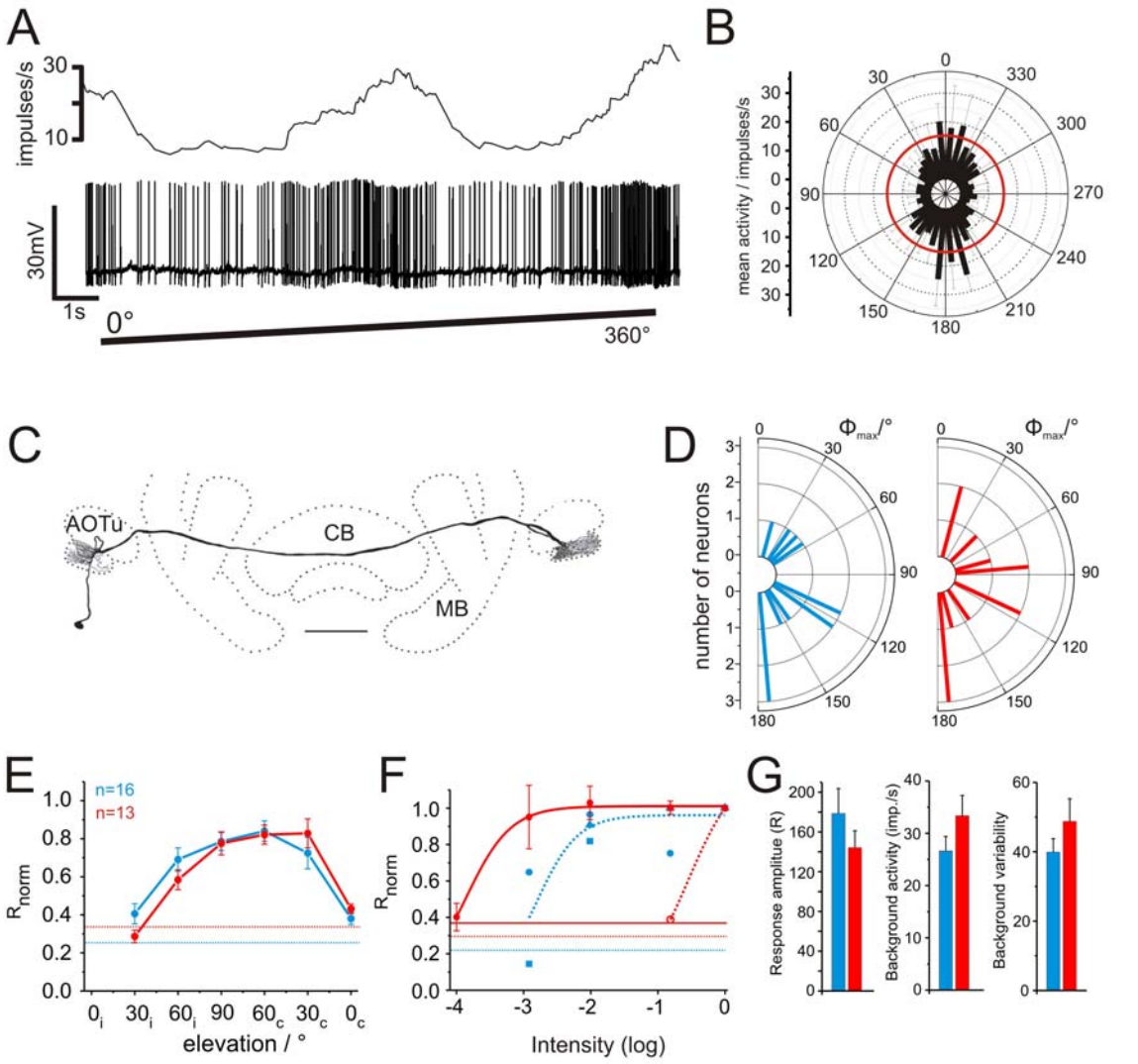

Figure 13. Intracellular recordings and anatomy of intertubercle neurons TuTu1 in the brain of the desert locust. **(A)** Spike train of a TuTu1 neuron during dorsal stimulation with a rotating polarizer (clockwise rotation, blue light, 450nm); lower trace: spike train; upper trace: mean spiking frequency (moving average of spike rate in 1s time window). **(B)** Circular diagram of mean frequencies of action potentials of the neuron in (A) plotted against *E*-vector orientation (n = 6, error bars = SD, bin size: 10°; Φ_{max} = 173.9°; Rayleigh test, $P < 10^{-12}$). Red circle indicates background activity. **(C)** Morphology of a TuTu1 neuron. AOTu: Anterior optic tubercle; CB; Central body; MB: Mushroom body. Modified from Pfeiffer et al. (2005). Scale bar: 200 µm. **(D)** Distribution of Φ_{max} orientations from TuTu1 neurons recorded from gregarious (left plot, blue bars, n=13) and solitarious (right plot, red bars, n=13) animals. In all cases stimulus elevation was 90°. The Φ_{max} -values refer to neurons with cell bodies in the left brain hemisphere. **(E)** Normalized response amplitudes R (R_{norm}) from TuTu1 neurons of 16 gregarious (blue) and 13 solitarious animals (red), plotted against the elevation of polarized-light stimuli. Ipsilateral (i) and contralateral (c) stimulations are defined with respect to the position of the soma of the recorded neuron. Data points are connected by lines for better visibility. R values and the mean background variability (dotted lines) of all recorded neurons are normalized to the maximum R value in the receptive field of each neuron. **(F)** Log I/response curves for R between 60° to 30° contralateral stimulation for gregarious locusts recorded during the day (blue, n=3), a solitarious locust recorded during the day (open red circles, dotted curve, n=1), and solitarious locusts recorded at night (red, n=6). In all cases, R values were normalized to the response amplitude R of log 0. Log I =0: 6.98 $\times 10^{13}$ photons cm^{-2} s^{-1}. Naka-Rushton fits. **(G)** Comparison of absolute response amplitude R in the center of the receptive field, background activity, and background variability of the recorded TuTu1 neurons between solitarious (red, n=13) and gregarious animals (blue, n=16). Error bars = standard error.

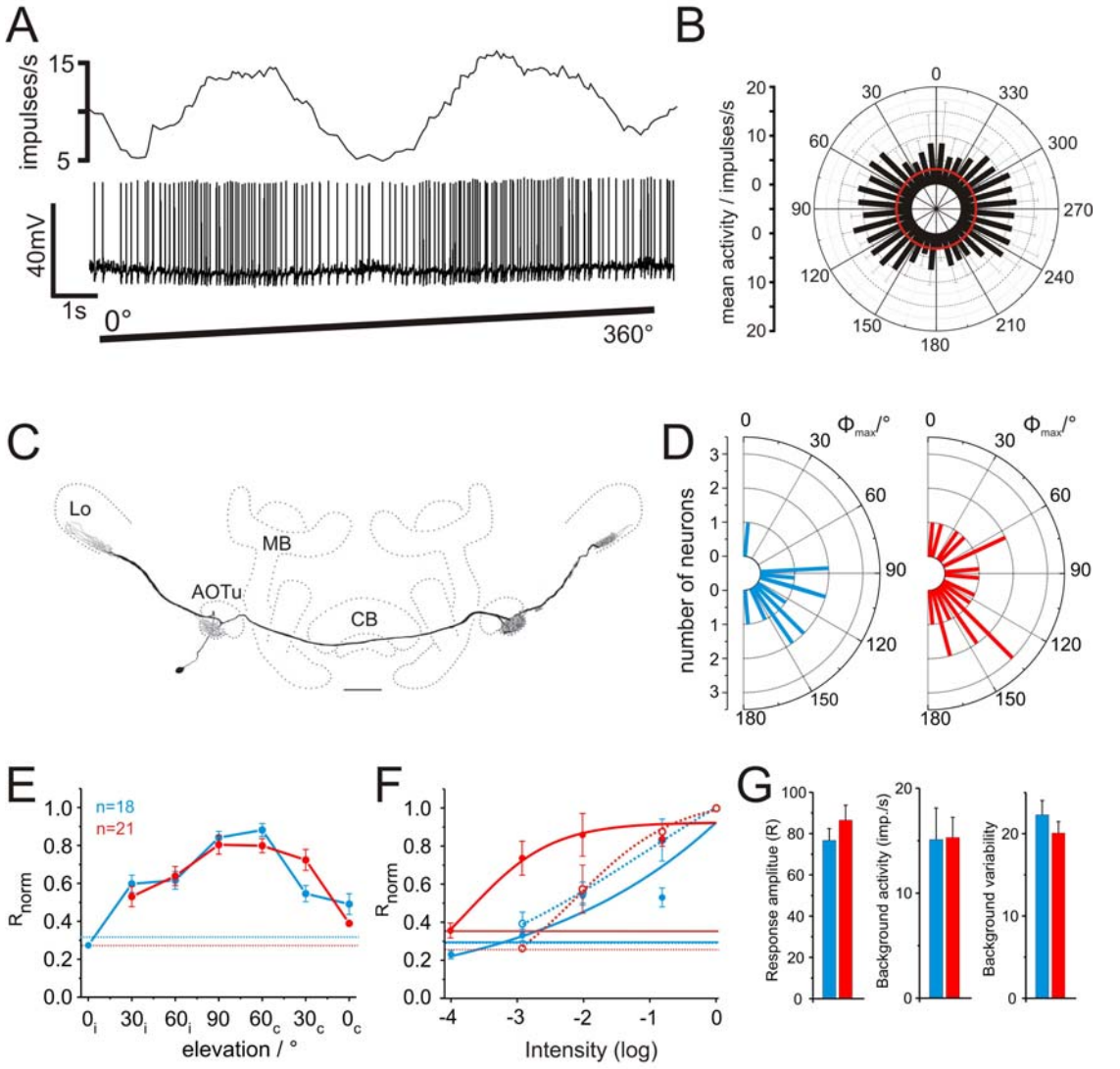

Figure 14. Physiology and morphology of LoTu1 neurons. **(A)** Responses of a LoTu1 neuron to a dorsally presented 360°-rotating polarizer (clockwise rotation, blue light, 450 nm); lower trace: spiking activity; upper trace: mean spiking frequency (moving average of spike rate in 1s time window). **(B)** Circular diagram of mean frequencies of action potentials of the neuron in (A) plotted against *E*-vector orientation (n = 6, error bars = SD, bin size: 10°; Φ_{max} = 93.9°; Rayleigh test, $P = 5.74^{-12}$). Red circle indicates background activity of the neuron. **(C)** Anatomy of a LoTu1 neuron. AOTu: Anterior optic tubercle; CB: Central body; MB: Mushroom body, Lo: Lobula. Modified from Pfeiffer et al. (2005). Scale bar: 200 µm. **(D)** Distribution of Φ_{max} orientations recorded from gregarious (left plot, blue bars, *n*=13) and solitarious (right plot, red bars, *n*=19) animals. The preferred directions of the neurons refer to neurons with cell bodies in the left brain hemisphere. **(E)** Relative response amplitudes *R* (R_{norm}) from 18 gregarious (blue) and 21 solitarious LoTu1 (red) neurons plotted against the elevation of polarized-light stimuli. Data points are connected by lines for better visibility. *R* values and mean background variability (dotted lines) of all recorded neurons are normalized to the maximum *R* value in the receptive field of each neuron. **(F)** Log I/response curves for R_{norm} (normalized to the *R* value at log 0 of each neuron) analyzed from elevations of 75° to 60° contralateral of gregarious locusts recorded during the day (ZT 0-12, blue, open circles, dotted curve, *n*=5), gregarious locusts recorded at night (ZT 12-24, blue, *n*=2) solitarious locusts recorded during the day (ZT 0-12, open red circles, dotted curve, *n*=3), and solitarious locusts recorded at night (ZT 12-24, red, *n*=11). Log I =0: 6.98×10^{13} photons cm^{-2} s^{-1}**.** Naka-Rushton fit**. (G)** Comparison of absolute response strength *R* in the center of the receptive field, mean background activity, and mean background variability of the LoTu1 neurons in gregarious (blue, *n*=18) and solitarious animals (red, *n*=21). Error bars= standard error.

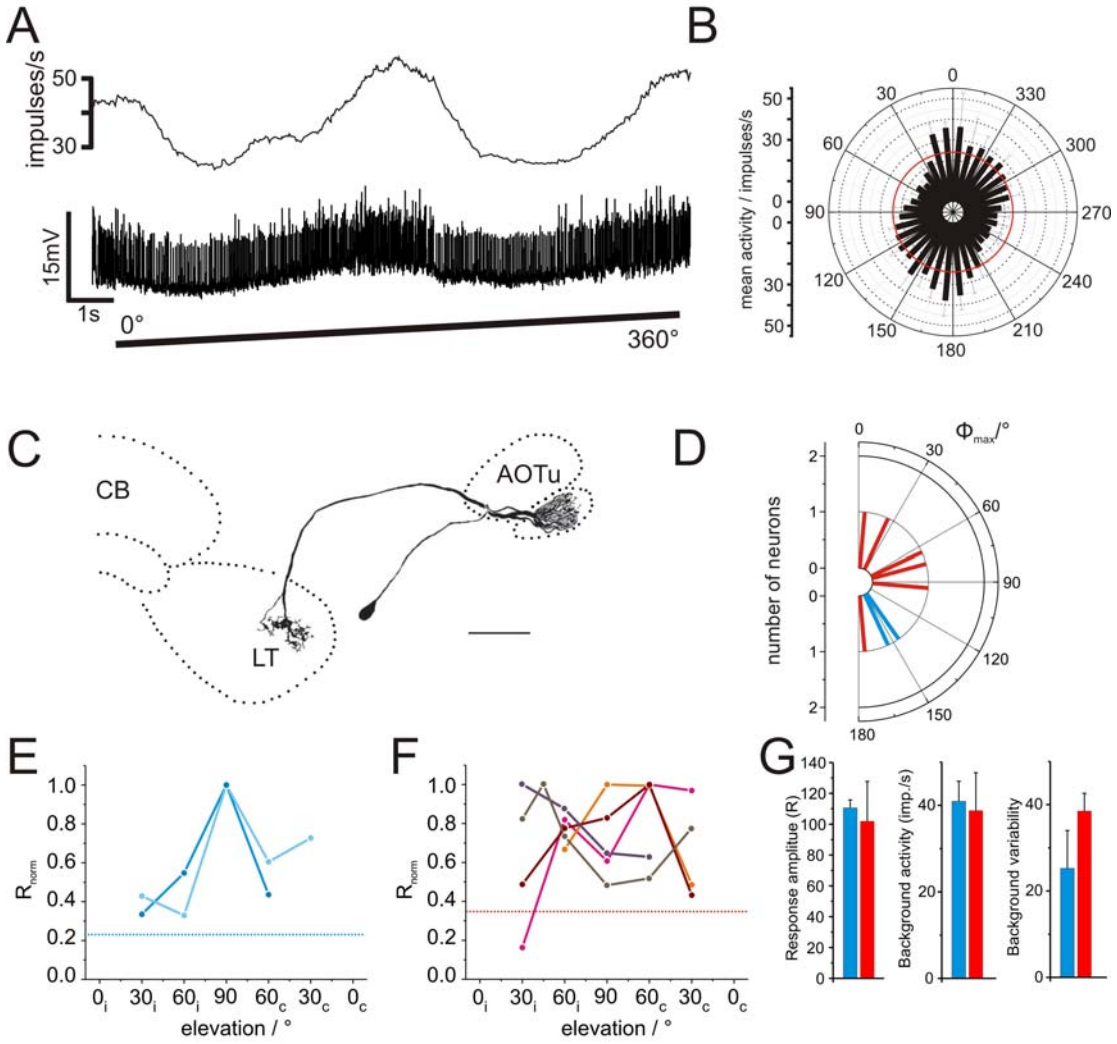

Figure 15. Comparison of TuLAL1a neurons between gregarious and solitarious locusts. **(A)** Neural activity of a TuLAL1a neuron during stimulation from dorsal direction with a rotating polarizer (clockwise rotation, blue light, 450nm); lower trace: spike train; upper trace: mean spiking frequency (moving average of spike rate in 1s time window). **(B)** Circular diagram of mean frequencies of action potentials of the neuron in (A) plotted against *E*-vector orientation (n = 4, error bars = SD, bin size: 10°; Φ_{max} = 158.2°; Rayleigh test, $P < 10^{-12}$). Red circle indicates background activity. **(C)** Morphology of a TuLAL1a neuron. AOTu: Anterior optic tubercle; CB; Central body; LT: Lateral triangle. Modified from Pfeiffer et al. (2005). Scale bar: 100µm. **(D)** Φ_{max}-distribution of seven TuLAL1a neurons recorded from gregarious (blue bars, *n*=2) and solitarious (red bars, *n*=5) animals. **(E, F)** R_{norm} of two gregarious (E) and five solitarious (F) animals plotted against the elevation of polarized-light stimuli. **(G)** Comparison of absolute response strength *R* in the center of the receptive field, mean background activity, and background variability of TuLAL1a cells in gregarious (blue, *n*=2) and solitarious locusts (red, *n*=5). Error bars= standard error.

'Thirteen TuTu1 neurons were recorded in solitarious animals. The neurons had a mean background activity of 33.3 ± 3.9 (SE) impulses per second and a mean background variability of 48.7 ± 6.5 (SE). Both the background activity and the background variability of TuTu1 neurons were not significantly different between solitarious and gregarious animals (Fig. 13G, two-tailed, t-test). In addition, the absolute response amplitude of TuTu1 neurons did not differ significantly between both morphs (Fig. 13G, two-tailed t-test). In contrast to Φ_{max} orientations

of TuTu1 neurons in gregarious locusts the preferred *E*-vector orientations of the neurons in solitarious animals were distributed more randomly (Fig. 13D, right circular plot). The receptive field structure of TuTu1 neurons in solitarious locusts is similar to that in gregarious animals with an eccentric center in the contralateral hemisphere. However, the receptive fields of TuTu1 neurons in solitarious animals are centered more laterally compared to those in gregarious animals and show the strongest response at an elevation of 30° (Fig. 13E). Response-intensity functions of TuTu1 neurons were analyzed in gregarious locusts at the subjective day (Zeitgeber Time: 0-12) and in the solitarious animals during their subjective day and during their subjective night (Zeitgeber Time: 12- 24). The response amplitude of the gregarious TuTu1 neurons was saturated between log I = 0 and log I =-2 and showed a sharp drop to background levels between log I =-2 and -3 (Fig. 13F, blue curve). A single TuTu1 neuron from a solitarious animal showed considerably lower sensitivity during the day and was unresponsive already at log I =-1 (Fig. 13F, red open circles, dotted line). TuTu1 neurons of solitarious locusts recorded at night (Fig. 13F, red curve, n=6) showed highest sensitivity and showed a decrease in response strength to background levels between log I = -3 and -4. Taken together, TuTu1 neurons from solitarious locusts recorded during the night are about 1 log unit more sensitive to polarized light than TuTu1 neurons in gregarious animals and about 4 log units more sensitive than TuTu1 neurons in solitarious animals recorded during the day.

LoTu1 neurons were recorded in 18 gregarious and 21 solitarious locusts. In gregarious animals LoTu1 neurons had a background activity of 15.1 ± 2.9 (mean ± SE) impulses per second and a mean background variability of 22.3 ± 1.68 (SE). The distribution of Φ_{max} orientations of the recorded neurons from gregarious animals showed clustering around 118° (Fig. 14D, left circular plot), but was statistically not different from randomness (Rao's spacing test, $P > 0.05$). The receptive field along the right-left meridian was eccentric and centered to the contralateral hemisphere (Fig. 14E, blue curve) with a peak response amplitude at an elevation of 60° contralaterally. In contrast to TuTu1 neurons, LoTu1 neurons had a narrower receptive field with a width of about 70°.

The mean background activity (15.3 ± 1.9 impulses/sec), background variability (20.1 ± 1.4) and the absolute response amplitude R in the center of the receptive field of LoTu1 in solitarious animals did not differ significantly from the background activity, variability, and response strength of the neurons in gregarious animals (Fig. 14G, two-tailed, t-test). However, the distribution of Φ_{max} orientations of the LoTu1 neurons in solitarious animals is more uniformly distributed than in gregarious animals (Fig. 14D, right circular plot). Interestingly, similar to the receptive fields of TuTu1 neurons, the receptive fields of LoTu1 neurons of solitarious animals were slightly shifted into the contralateral visual field. The strongest response occurred at an elevation between the zenith and 60° contralaterally. Therefore, response-intensity functions of LoTu1 neurons were analyzed at elevations of 75° to 60° contralaterally. Five LoTu1 neurons of gregarious locusts that were recorded during the day (Fig. 14F, open blue circles, dotted blue curve) and two LoTu1 cells of gregarious animals that were recorded at night (Fig. 14F, blue circles, solid blue curve) showed similar intensity-response curves that gradually decreased to background levels between log I= 0 and log I = -3. Recordings from three LoTu1 neurons in solitarious animals during the day had a sensitivity curve similar to the LoTu1 neurons of gregarious animals (Fig. 14F, red open circles, dotted red curve). In contrast, the intensity response curve of LoTu1 neurons from solitarious animals recorded during the night (n=11, red circles, red solid curve) was shifted to lower intensities by 1-2 log units and only decreased to background levels between log I=-2 and -4. Taken together, LoTu1 neurons in solitarious locusts recorded during the night are about 1-2 log units more sensitive than LoTu1 neurons from gregarious animals and from solitarious animals recorded during the day.

Owing to the small neurites of TuLAL1a neurons, recordings of these neurons are relatively difficult and, therefore, only seven recordings were successful. The receptive fields of two TuLAL1a neurons were analyzed in gregarious locusts. Background activities of the neurons ranged from 36.2 to 45.5 impulses per second and the background variability ranged from 16.5 to 34. The receptive fields of both neurons were zenith-centered and quite narrow (about 60°) (Fig. 15E). The Φ_{max} orientation of both neurons was around 150° whereas the Φ_{max} orientations in five TuLAL1 neurons from solitarious animals were distributed randomly (Fig. 15D). The neurons in solitarious animals had a mean background activity of 38.7 ± 8.8 (SE) impulses/s and a mean background variability of 38.5 ± 4.1 (SE). No differences were observed in response strength, background activity, and background variability between solitarious and gregarious locusts (Fig. 15G, two-tailed t-test). The receptive fields of the solitarious TuLAL1a neurons varied considerably in bilateral size and position and had centers in the contralateral or ipsilateral hemisphere (Fig. 15F). Some receptive fields were broader than those of the two neurons from gregarious animals. Considering the total number of TuLAL1a neurons in each brain hemisphere (about 20-30 TuLAL1a neurons) a meaningful comparison of these cell types in gregarious and solitarious animals will have to await further data. Intensity-response functions have not been determined for TuLAL1a neurons.

An interesting fact that was mentioned already in the last progress report is the difference in the shape of the intensity-response curves between LoTu1 and TuTu1 neurons. Whereas TuTu1 neurons responded above threshold levels independently of light intensity (Fig. 13F), signals in LoTu1 cells were strongly dependent on light intensity (Fig. 14F). To analyze this difference in more detail we compared background activities and absolute response amplitudes of TuTu1 and LoTu1 neurons that were recorded during the day with those that were recorded at night (Fig. 16A,C). Whereas no significant differences of the background activity and the response amplitude occurred in TuTu1 neurons recorded during the day and at night (two-tailed t-test, Fig. 16A), the absolute response amplitude of LoTu1 neurons was significantly higher ($P< 0.01$, two-tailed. t-test) at night than during the day (Fig. 16C). To further investigate this effect, we stimulated LoTu1 and TuTu1 neurons in gregarious and solitarious animals with bright polarized white light and blue polarized light from dorsal direction and compared the response strength as well as the ratio between the response amplitude and background firing rates. The ratio between response amplitude *R* and background activity provides an estimate of the information content of the frequency modulations during rotation of the polarizer (Heinze et al. 2009). In TuTu1 neurons (n=2) we did not find significant differences in *R* and R/background activity between polarized blue light stimulation and bright polarized white light (Fig. 16B). Interestingly LoTu1 neurons stimulated with blue polarized light showed a significantly higher response amplitude than LoTu1 neurons stimulated with bright polarized white light (n=5, Fig. 16D). In two of the neurons the sinusoidal modulation of spike frequency during rotation of the polarizer was completely abolished and the neurons were totally inhibited (P>0.01, one-tailed, t-test). As a result of this decreased response amplitude *R*, the ratio between *R* and background firing rate was also significantly different between stimulation with polarized blue light and bright polarized white light (P>0.05, one-tailed, t-test).

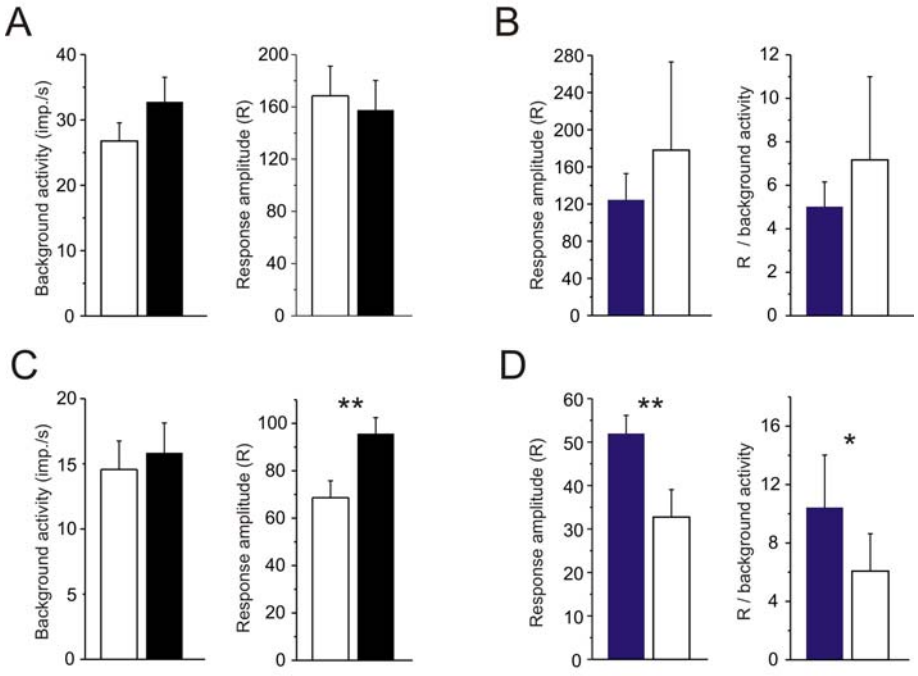

Figure 16. Physiology of polarization-sensitive interneurons of the anterior optic tubercle at night and during the day (A, C) and when stimulated with polarized blue vs. white light (B, D). **(A)** Background activity and absolute response amplitude *R* during stimulation with a rotating polarizer (blue light, 450 nm) of TuTu1 neurons recorded during the day (ZT 0-12; white bars, *n*=15) and TuTu1 neurons recorded at night (ZT 12-24; black bars, *n*=14). **(B)** Response amplitude (R) and the ratio of response amplitude and background firing rate of two TuTu1 neurons stimulated with polarized blue light (blue bars; I=30.82 μW/cm²) and high-intensity polarized white light (white bars; I= 39174.32 μW/cm²). **(C)** Mean background activity and absolute response amplitude *R* during stimulation with a rotating polarizer (blue light, 450 nm) in the center of the receptive field of LoTu1 neurons recorded during the day (ZT 0-12; white bars, *n*=19) and neurons recorded at night (ZT 12-24; black bars, *n*=19). **(D)** Response amplitude and ratio of response strength and background activity of LoTu1 neurons (*n*=5) during stimulation with polarized blue light from dorsal direction (blue bars; I=30.82 μW/cm²) and high-intensity polarized white light (white bars; I= 39174.32 μW/cm²). Error bars: standard error; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$; no asterisk means no significant differences.

Taken together, the response intensity curves signaling sky polarization are shifted in solitarious locusts to higher sensitivities at night. This is seen in both LoTu1 and TuTu1 neurons and can be interpreted as an adaptation to navigation of solitarious locusts under low light conditions. In addition, TuTu1 neurons have saturated and constantly high response amplitudes above a certain threshold, whereas LoTu1 neurons both in solitarious and gregarious animals show reduced response amplitudes at brightest light intensities, when the sun is high in the sky and the overall degree of sky polarization is low. LoTu1 neurons are therefore adapted in both morphs to signal polarized light at low intensity light conditions, probably during sunset and sunrise, when the degree of polarization in the sky is high. Comparison of the absolute sensitivities in the two dorsal rim photoreceptors and TuTu1 and LoTu1 neurons further indicates a 1-2 log unit higher sensitivity in the interneurons compared to the photoreceptors. This is likely caused by pooling of inputs from many dorsal rim photoreceptors to the tubercle neurons and has similarly also been noted in the cricket polarization vision system (Labhart et al. 2001).

Differences between solitarious and gregarious locusts have recently also been reported for physiological parameters of a looming-sensitive visual interneuron (Rogers et al., 2010). Overall

the neuron in gregarious animals responded more strongly to potentially threatening looming stimuli and showed less habituation to repetitive stimulation. This has been interpreted as an adaptation to different predators with different approach strategies in the two morphs. We can now add that neurons of the sky compass navigation system are more sensitive in solitarious animals, however apparently only during their active phase at night, which can be regarded as another important adaptation in the nocturnal morph.

V. GENERAL CONCLUSION

As the result of using a wide variety of model organisms and approaches, the three research groups have progressed significantly in this sixth and final six-month period of the AFOSR contract in their investigations of dim-light vision. Some projects are still in progress whereas others are nearing completion. During the funding period we have accumulated a large amount of data, both behavioural and physiological, that has so far led to 6 published papers (shown in red below) and to several others that are in preparation. Our work has led to considerable advances in our understanding of how well nocturnal insects (bees, dung beetles and locusts) navigate visually and which neural and optical mechanisms are responsible, advances which have been detailed in all 6 reports and which are, or soon will be, published. All three laboratories are deeply grateful for the support they have received from the AFOSR.

VI. REFERENCES

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